1: Lett Appl Microbiol 1992 Jun;14(6):250-4

An improved method for rapid purification of covalently closed circular plasmid DNA over a wide size range.

Azad AK, Coote JG, Parton R.

Department of Microbiology, University of Glasgow, UK.

An improved method has been developed for the large-scale purification of covalently closed circular (CCC) plasmid DNA molecules of sizes ranging from 4.3 to 73 kb. This protocol uses an alkaline-lysis procedure followed by acid-phenol extraction but with several modifications to previously reported methods. The principal modification is the replacement of NaCl by MgCl2 in the extraction buffer to improve yield and to remove chromosomal and other non-CCC plasmid DNA. Plasmid DNA can be purified in less than 1 h and used successfully in restriction enzyme analysis and cloning experiments.

PMID: 1368369 [PubMed - indexed for MEDLINE]

1: J Chromatogr A 1998 May 8;806(1):31-45 Related Articles, Bool

Preparative purification of supercoiled plasmid DNA using anion-exchange chromatography.

Prazeres DM, Schluep T, Cooney C.

Centro de Engenharia Biologica e Quimica, Instituto Superior Tecnico, Lisbon, Portugal.

Large scale manufacturing of gene vectors such as plasmid DNA is an important issue in gene therapy. Anion-exchange chromatography is fundamental in the downstream processing of plasmids both as a process and analytical technique. This work reports the use of Q-Sepharose columns (1, 1 and 40 ml) for the preparative purification of plasmid pUC18. NaCl gradient elution enabled the isolation of supercoiled plasmid from low-M(r) RNA, cDNA and plasmid variants. A compact covalently closed, supercoiled form of denatured plasmid carrying large stretches of single-stranded DNA was identified as one of the major contaminants. Anion-exchange HPLC on a Poros QE 20 column was used to quantify plasmid yield. Supercoiled plasmi was recovered in a single fraction with a 62 +/- 8% yield. Loadings higher than 40 micrograms/ml gel could be used but at the expense of a loss of resolution between open circular and supercoiled forms. Plasmid quality was evaluated by gel electrophoresis, restriction analysis, transformation experiments and protein assays.

PMID: 9639879 [PubMed - indexed for MEDLINE]

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1: Biotechniques 1996 Jul;21(1):92-4, 96-9 Related Articles, Book

Comparison of plasmid DNA preparation methods for direct gene transfer and genetic immunization.

Davis HL, Schleef M, Moritz P, Mancini M, Schorr J, Whalen RG.

Loeb Medical Research Institute, Ottawa Civic Hospital, ON, Canada.
hdavis@civich.ottawa.on.ca

Plasmid DNA is widely used for direct gene transfer in animals to study gene therapy, gene regulation, drug delivery and genetic immunization. Here we compare cesium chloride and anion-exchange purified plasmid DNA for direct gene transfer in mouse muscle and show no differences in efficiency o transfection with reporter genes or in humoral response to DNA-based immunization.

Publication Types:
- Technical report

PMID: 8816242 [PubMed - indexed for MEDLINE]

1: Biotechniques 1996 Mar;20(3):492-7

Preparation of pure plasmid or cosmid DNA using single-strand affinity matrix and gel-filtration spin columns.

Pham TT, Chillapagari S, Suarez AR.

CLONTECH Laboratories, Inc., Palo Alto, CA, USA.

A rapid method has been developed for ultrapure plasmid or cosmid DNA isolation from ten-mL to several hundred-mL cultures of Escherichia coli (midi to maxi prep). A cleared lysate is prepared by alkaline lysis, followed by a quick alcohol precipitation step. Denatured bacterial DNA and RNA having at least 20 nucleotides of single-stranded regions are removed from th supercoiled plasmid by binding strongly to the single-strand affinity matrix (SSAMTM). Plasmid DNA is then effectively purified on a gel-filtration spir column to remove SSAM, proteins, small RNA and salts. This method produces consistent yields of high-quality plasmids that are suitable for use i many molecular biology applications. In addition, recombinant cosmids of approximately 46 kb can be purified intact, free of chromosomal DNA.

PMID: 8679211 [PubMed - indexed for MEDLINE]

1: Anal Biochem 1995 Sep 1;230(1):130-4

A filtration method for plasmid isolation using microtiter filter plates.

Ruppert A, Szalay B, van den Boom D, Horst G, Koster H.

Faculty of Chemistry, Department of Biochemistry and Molecular Biology, University of Hamburg, Germany.

A method for the simultaneous isolation of plasmid DNA from as many as 9(Escherichia coli clones in less than 2 h is described. It is based on a modified version of the alkaline lysis procedure originally described by Birnboim and Doly (Nucleic Acids Res. 7, 1513-1523, 1979). The handling of DNA samples is facilitated by the use of microtiter plates with membrane filter bottoms. All centrifugation steps are replaced by filtrations ("the filtration method"). The yield of plasmid DNA from 0.35 ml of an overnight culture is sufficient for restriction analysis of the plasmid clones. Up to 400 nucleotide: readable sequences could be obtained in cycle sequencing reactions with an unmodified sequencing protocol on an automated ABI sequencer.

PMID: 8585608 [PubMed - indexed for MEDLINE]

1: FEMS Microbiol Lett 1995 May 15;128(3):307-13 Related Articles, Books, LinkO

Plasmid and chromosomal DNA recovery by electroextraction o cyanobacteria.

Moser D, Zarka D, Hedman C, Kallas T.

Department of Biology and Microbiology, University of Wisconsin-Oshkosh USA.

High voltage electroporation has been investigated as a method for rapid recovery of plasmid and chromosomal DNA from the cyanobacteria Nostoc PCC 7121, Synechococcus PCC 7002, and Anabaena PCC 7120. Pulses of 1 kV/cm and higher applied to concentrated Nostoc cells carrying a shuttle plasmid (pRL25) resulted in copious release of nucleic acids and phycobiliproteins into the suspending medium. Small portions of these supernatants, when electroporated with Escherichia coli, gave rise to hundrec of E. coli transformants which contained pRL25. Electroporation of Synechococcus carrying plasmid pAQE19 did not cause detectable release o macromolecules but did reveal a low-level, voltage independent 'leakage' of pAQE19 into the medium. Electroextraction of Nostoc or Anabaena followe by addition of E. coli and delivery of a second high-voltage pulse permitted direct, one-cuvette transfer of shuttle plasmids from these cyanobacteria into E. coli. Electroextraction of single cyanobacterial colonies, as shown for Nostoc, also released sufficient chromosomal DNA for amplification of specific sequences by the polymerase chain reaction.

PMID: 7781980 [PubMed - indexed for MEDLINE]

1: Hum Gene Ther 1995 May;6(5):565-73

Related Articles, Books, LinkO

Comment in:

- Hum Gene Ther. 1995 May;6(5):549-50
- Hum Gene Ther. 1995 May;6(5):551-2

Cancer gene therapy using plasmid DNA: purification of DNA for human clinical trials.

Horn NA, Meek JA, Budahazi G, Marquet M.

Vical Inc., San Diego, CA 92121, USA.

A production method has been developed for the purification of pharmaceutical-grade plasmid DNA for in vivo gene therapy. This method has been applied to the purification of VCL-1005, which is a eukaryotic plasmid expression vector that codes for the production of the HLA-B7 protein. Purified VCL-1005 is formulated with a cationic lipid and injected directly into established tumors of HLA-B7-negative patients with advanced cancers to heighten the patient's immune response against the cancer. The purification of pharmaceutical-grade plasmid DNA requires the development of highly reproducible and scaleable processing methods that meet regulatory standards similar to those required for the manufacture of recombinant protei pharmaceuticals. Defined pharmaceutical standards of purity, potency, efficacy, and safety are routinely met by the process described in this study. The scaleable purification method described here is a combination of highly reproducible unit operations; alkaline lysis, precipitation, and size-exclusion chromatography. The advantages over existing DNA purification methods include improved plasmid purity and the elimination of undesirable process additives such as toxic organic extractants and animal-derived enzymes. The overall process yield of purified plasmid DNA from fermentation through final column purified product is greater than 50%. Contaminating Escherichi coli DNA levels are reproducibly below 1% as measured by Southern analysis. Endotoxin levels are less than 0.03 endotoxin units/micrograms plasmid DNA and residual protein is undetectable. This process was used to produce 100 mg of VCL-1005 for use in an active clinical protocol.

PMID: 7578394 [PubMed - indexed for MEDLINE]

1: Hum Gene Ther 1995 Mar;6(3):317-23

Bacterial lipopolysaccharide copurifies with plasmid DNA: implications for animal models and human gene therapy.

Wicks IP, Howell ML, Hancock T, Kohsaka H, Olee T, Carson DA.

Department of Medicine, University of California, San Diego, La Jolla 9209: 0663, USA.

During the course of gene therapy experiments in rodents, using intramuscul injections of plasmid DNA derived from Escherichia coli, we noted dose-related toxicity. This observation prompted a search for possible contaminan of DNA samples. We used the highly specific and sensitive limulus amoebocyte lysate assay (LAL), to monitor endotoxin bioactivity in DNA samples, and found plasmid DNA derived from standard E. coli bacterial strains, using traditional DNA isolation protocols, to be heavily contaminate with endotoxin, or lipopolysaccharide (LPA). Standard DNA isolation procedures resulted in the copurification of up to 500 micrograms/ml of LPS LPS is a potent inducer of cytokines and other inflammatory mediators, and may complicate the use of naked DNA in gene therapy. The copurification o endotoxin with plasmid DNA also has important implications for in vitro transfection studies and microinjection of DNA into embryos. A simple and efficient protocol to reduce LPS contamination of plasmid DNA was developed. The conversion of intact bacteria to spheroplasts prior to the isolation of plasmid DNA, incubation with lysozyme, treatment with the detergent n-octyl-beta-D-thioglucopyranoside (OSPG) and polymyxin-B (PMB) chromatography, allowed the isolation of plasmid DNA containing less than 50 ng/ml LPS. This represents a 10,000-fold reduction in LPS contamination, compared to conventional methods of plasmid DNA purification, avoids potentially toxic reagents such as ethidium bromide, and produces a higher yield of plasmid DNA.

PMID: 7779915 [PubMed - indexed for MEDLINE]

1: Biotechniques 1994 Mar;16(3):514-9

Related Articles, Boo

Two-minute miniprep method for plasmid DNA isolation.

Tarczynski MC, Meyer WJ, Min JJ, Wood KA, Hellwig RJ.

5 Prime-->3 Prime, Inc., Boulder, CO 80303.

An extremely rapid method, INSTA-PREP, has been developed to prepare plasmid DNA from 1 to 3 mL miniprep Escherichia coli bacterial cultures. Direct extraction of plasmid DNA from E. coli bacterial cells is achieved by two-phase solution consisting of phenol-chloroform-isoamyl alcohol and water or buffer with efficient separation of the phases by centrifugation in th presence of the INSTA-PREP gel barrier material. Processing time, from E. coli culture to usable plasmid DNA, is two minutes or less per sample. Supercoiled plasmid DNA yields ranged from 3 to 10 micrograms per mL of culture depending on plasmid copy number. Plasmid DNAs prepared by INSTA-PREP were analyzed and are suitable for use in molecular biology procedures including restriction digestion, ligation with T4 DNA ligase, bacterial transformation, PCR, cultured cell transfection and T7 DNA polymerase or thermostable DNA polymerase-mediated dideoxynucleotide sequencing.

PMID: 8185927 [PubMed - indexed for MEDLINE]

1: Biotechniques 1994 Mar;16(3):460-3 Related Articles, Books, LinkO

Large-scale supercoiled plasmid preparation by acidic phenol extraction.

Wang Z, Rossman TG.

Norton Nelson Institute of Environmental Medicine, New York, NY.

A novel method for large-scale plasmid preparation is described. Crude extracts are subjected to acidic phenol extraction to remove any contaminant: present in the aqueous phase. The supercoiled plasmid DNA, which preferentially remains in the organic phase and inter-phase, is extracted back into the aqueous phase with 1.5 M TRIZMA base, from which it is precipitated. The resultant plasmid DNA is highly pure and satisfactory for any subsequent procedures. The method is extremely economical and takes only 3-4 h.

Publication Types:
- Technical report

PMID: 8185920 [PubMed - indexed for MEDLINE]

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1: Anal Biochem 1993 Aug 1;212(2):394-401

A modified alkaline lysis method for the preparation of highly purified plasmid DNA from Escherichia coli.

Feliciello I, Chinali G.

CEINGE, Dipartimento di Biochimica e Biotecnologie Mediche, IIa Facolta di Medicina e Chirurgia, Universita di Napoli, Italy.

We have developed a very efficient and rapid method for the preparation on small or large scale of highly purified plasmid DNA from Escherichia coli. The procedure consists of five steps: (1) cell lysis by NaOH-SDS, (2) precipitation of cell lysate with 2 M potassium acetate-1 M acetic acid, (3) precipitation of the resulting supernatant with isopropanol, (4) treatment of the precipitate with RNase, and (5) a second isopropanol precipitation. The new procedure yields a plasmid DNA that is more than 90% in the supercoiled form and virtually free from proteins, RNA, and chromosomal DNA. We have thoroughly tested the method in the preparation of several thousand samples of different plasmids from various E. coli strains. We foun that it consistently produced samples of plasmid DNA suitable for all routine uses such as restriction analysis, sequencing, and preparation of DNA probes for cloning and hybridization experiments. Moreover, plasmids purified by this procedure could fully replace plasmids purified on CsCl gradients for more demanding tasks such as the in vitro synthesis of RNA probes by phage RNA polymerases, the generation of deletion mutants with exonuclease III, and the transfection of mammalian cells by the calcium phosphate coprecipitation method, as tested on human fibroblasts and on CV-1 cells.

PMID: 8214582 [PubMed - indexed for MEDLINE]

1: Biotechnol Appl Biochem 1992 Oct;16(2):211-5

A procedure for large-scale plasmid isolation without using ultracentrifugation.

Chakrabarti A, Sitaric S, Ohi S.

Department of Biochemistry, School of Medicine, Meharry Medical College, Nashville, Tennessee 37208.

An expedient procedure for large-scale plasmid isolation from Escherichia coli strains without using ultracentrifugation or special setups or reagents is described. The protocol, which utilizes a modified alkaline extraction procedure as well as differential precipitations by isopropanol and lithium chloride, is simple and rapid and yet produces plasmid DNA with a yield of about 2 mg/liter culture. The isolated plasmids consisted of mostly monomeric and dimeric covalently closed circular DNA. The plasmids could be digested by various restriction endonucleases and were compatible with gene cloning, transfection-gene expression, and viral production.

PMID: 1333773 [PubMed - indexed for MEDLINE]

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1: Genet Anal Tech Appl 1991 May;8(3):107-10 Related Articles, Bool

An improved and rapid procedure for isolating RNA-free Escherichia coli plasmid DNA.

He M, Kaderbhai MA, Adcock I, Austen BM.

Department of Surgery, St. George's Hospital Medical School, London, UK.

We describe a simple, rapid, and inexpensive procedure for the isolation of plasmid DNA in high yields from Escherichia coli cultures. The procedure entails two main steps, which involve treating intact bacterial cells with phenol/chloroform in the presence of Triton X-100 and LiCl followed by polyethylene glycol precipitation. Plasmid DNA preparations isolated by this method are highly pure and virtually devoid of RNA. The DNA is suitable substrate for restriction mapping, DNA-modifying enzymes, and in vitro transcription with SP6 and T7 RNA polymerases.

PMID: 1712213 [PubMed - indexed for MEDLINE]

Display Abstract Save Text Order Add to Clipboard

1: Biotechniques 1990 Jul;9(1):19-20, 22, 24

Large-scale isolation of plasmid DNA using cetyltrimethylammonium bromide.

Ishaq M, Wolf B, Ritter C.

Dept. of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia 19104-6049.

A rapid procedure for the large-scale isolation of plasmid DNA is described. The method utilizes cetyltrimethylammonium bromide to precipitate the plasmid following extraction of DNA by lysozyme digestion and boiling. Th plasmid is then purified by passing through the spin column pZ523. The purity and yield of the plasmid obtained with this method is similar to that isolated by cesium chloride-ethidium bromide gradient centrifugation. The method does not involve any phenol-chloroform extractions and takes five to six hours for completion after growth of the bacterial cells. The plasmid obtained is amenable to digestion with various restriction endonucleases, can be used for cloning with high efficiency and is also suitable as template for dideoxy sequencing.

PMID: 2168186 [PubMed - indexed for MEDLINE]

1: Biotechniques 1988 Oct;6(9):834, 837-8

A simple method for the preparation of the covalently closed circular form of plasmid DNA.

Le Brun JJ, Rentier-Delrue F, Mercier L.

Dept. of Botany, University of Maryland, College Park 20742.

We describe a simple, rapid, inexpensive method for isolation of covalently closed circular plasmid DNA. The method involves the electrophoresis of crude DNA preparations in an agarose gel, electrotransfer onto a dialysis membrane and elution of the highly purified circular covalently closed plasmid DNA. Native and recombinant plasmid DNA have been purified by this method and shown to be suitable for restriction enzyme digestion and transformation of bacteria. The yield of this rapid purification procedure makes it a good alternative method to standard centrifugation in cesium chloride ethidium bromide gradients.

PMID: 3078644 [PubMed - indexed for MEDLINE]

1: Anal Biochem 1987 Oct;166(1):188-93

Purification and separation of various plasmid forms by exclusion chromatography.

Moreau N, Tabary X, Le Goffic F.

CNRS, CERCOA 2 a 8, Thiais, France.

A chromatographic method for the rapid isolation of preparative amounts of plasmid DNA without the use of cesium chloride centrifugation is described. The protocol uses the alkaline extraction procedure and an exclusion column of Fractogel TSK 75S. From a clear lysate it is possible to obtain plasmid DNA completely free of proteins, RNA, and chromosomal DNA. From partially purified plasmid the procedure allows the separation of the different forms. This technique was successfully applied to different plasmids ranging in size from 2.9 to 17.5 MDa. It is a preparative method yielding easily 500 micrograms of pBR322 from 1 liter of amplified culture. The plasmid is suitable for topoisomerase I, topoisomerase II, and EcoRI assays.

PMID: 2445230 [PubMed - indexed for MEDLINE]

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1: Anal Biochem 1987 Feb 1;160(2):332-6 Related Articles, Book

A procedure for large-scale isolation of RNA-free plasmid and phage DNA without the use of RNase.

Lev Z.

A preparative procedure for the large-scale isolation of plasmid DNA withou the use of RNAse is described. Crude plasmid DNA is prepared using a standard boiling method. High-molecular-weight RNA is removed by precipitation with LiCl, and low-molecular-weight RNA is removed by sedimentation through high-salt solution. The procedure is inexpensive, rapic simple, and particularly suitable for processing several large-scale preparations simultaneously. A similar procedure has been developed for preparation of lambda-phage DNA.

PMID: 2437820 [PubMed - indexed for MEDLINE]

[redacted]

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1: Mol Gen Mikrobiol Virusol 1985 Sep;(9):44-7 Related Articles, Boo

[Purification of plasmid DNA by chromatographic methods].

[Article in Russian]

Naumov GN.

Chromatographic methods have been used to purify the DNA of plasmid RP DNA was purified in two stages. DNA was precipitated by ethanol and separated from RNA and proteins in Sepharose 4B column after lysis of plasmid containing cells by alkaline solution of sodium dodecylsulphate. Separation of the total DNA preparation and isolation of plasmid DNA was achieved at the second stage by chromatography on the hydroxyapatite column. The resulting purified plasmid DNA was free of RNA, protein and linear fragments of chromosomal DNA. The plasmid DNA kept intact native structure and possessed the transforming activity. The DNA of RP1 yield aft purification by the described technique presented 70-80 micrograms per g of wet biomass.

PMID: 3916234 [PubMed - indexed for MEDLINE]

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1: Anal Biochem 1983 Sep;133(2):265-70

Related Articles, Bool

A simple procedure for large-scale preparation of pure plasmid DNA free from chromosomal DNA from bacteria.

Mukhopadhyay M, Mandal NC.

A very simple, inexpensive procedure for preparing pure plasmid DNA from bacteria is described. In this method, lysozyme-induced spheroplasts are mac in presence of 833 micrograms/ml of ethidium bromide which are then lysed by a mixture of Brij 58 and sodium deoxycholate, and the lysate is centrifuge at 48,000 g for 25 min whereby about 99.9% of total chromosomal DNA is pelleted. From the supernatant containing plasmid DNA, the proteins are removed by phenol extraction and the major part of RNA by CaCl2 precipitation, and finally the small amount of residual RNA is removed by RNase treatment. The average yield of pBR322 DNA from 1 liter of amplifie culture by this procedure is 2 to 2.5 mg and the preparation is highly pure, containing only about 0.005% of total yield as chromosomal DNA contaminant. Moreover, the substrate activity and the transforming ability of the plasmid DNA prepared by this method remain unaffected.

PMID: 6356982 [PubMed - indexed for MEDLINE]

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1: Mol Biol Rep 1983 Aug;9(3):191-5

Related Articles, Bool

A method for the purification of E. coli plasmid DNA by homogeneous lysis and polyethylene glycol precipitation.

Pulleyblank D, Michalak M, Daisley SL, Glick R.

A procedure is described for the isolation and purification of E. coli plasmid DNA by polyethylene glycol precipitation. The method is rapid, simple, inexpensive and amenable to both small and large scale manipulation. This procedure involves lysis of bacterial cells by treatment with pronase in sodium dodecyl sulfate, removal of chromosomal DNA by centrifugation, precipitation of residual nucleic acids with polyethylene glycol and removal of RNA by precipitation with LiCl. Plasmid DNA purified as described is pure enough for restriction endonuclease analysis, for use as a vector for the cloning of cDNA or synthetic DNA, or for use as a template in an E. coli transcription-translation cell-free system.

PMID: 6355817 [PubMed - indexed for MEDLINE]

[redacted] PubMed

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1: Prep Biochem 1983;13(2):161-6 Related Articles, Bool

Large scale purification of plasmid DNA.

Bachvarov DR, Ivanov IG.

A simple and rapid procedure for large scale purification of plasmid DNA is described. The procedure consists of two main steps: 1. Alkaline extraction o plasmid DNA (by a slight modification of the method of Birnboim and Doly (1)) and 2. Purification of the crude extract by hydroxyapatite chromatography. The plasmids obtained are biologically active and can be used in gene manipulation experiments.

PMID: 6348728 [PubMed - indexed for MEDLINE]

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1: Eur J Biochem 1978 Nov 2;91(1):303-10 Related Articles, Boo

Rapid purification of plasmid DNAs by hydroxyapatite chromatography.

Colman A, Byers MJ, Primrose SB, Lyons A.

A method is described for the rapid preparation of plasmid DNAs of molecular weight up to 14 X 10(6). This method involves the chromatography, at room temperature, of bacterial cleared lysates on hydroxyapatite in the presence of high concentrations of phosphate and urea. All detectable protein and RNA contamination of plasmid DNA is removed by this procedure and the conformation of the plasmid DNA is unaffected. Less than 0.5% chromosomal DNA is present in the purified preparation and even this can be removed if necessary by a simple extention of the procedure to include a heat-denaturation step. The method is extremely rapid and amenable to large-scale plasmid preparation; 5 mg ColE1 DNA have been purified within 40 min. The yield of plasmid DNA is similar to that obtained with the conventional dye-centrifugation technique, however the purity is greater.

PMID: 363426 [PubMed - indexed for MEDLINE]

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1: Biochim Biophys Acta 1975 Apr 2;383(4):457-63 Related Articles, Boo

A simple method for the preparation of large quantities of pure plasmid DNA.

Humphreys GO, Willshaw GA, Anderson ES.

Polyethylene glycol quantitatively precipitates plasmid DNA of molecular weight 6-123-10-6, from cleared lysates of plasmid-carrying bacterial strains After resuspension and density-gradient centrifugation of the precipitated DNA, it is unchanged in length and in transformation efficiency for Escherichia coli K12. Plasmid DNA can be easily prepared in large quantitie by including a polyethylene glycol precipitation step in standard plasmid isolation procedures.

PMID: 1092355 [PubMed - indexed for MEDLINE]

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1: Biotechniques 1995 Dec;19(6):884-90

Related Articles, Books, LinkO

Large-scale purification of plasmid DNA for biophysical and molecular biology studies.

Baumann CG, Bloomfield VA.

University of Minnesota, St. Paul, MN, USA.

PMID: 8747649 [PubMed - indexed for MEDLINE]

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1: Biotechniques 1998 Mar;24(3):438-44 Related Articles, Boo

Erratum in:
- Biotechniques 1998 Jun;24(6):992

Bacterial growth medium that significantly increases the yield o recombinant plasmid.

Duttweiler HM, Gross DS.

Louisiana State University Medical Center, Shreveport, USA.

Isolation of plasmid DNA from Escherichia coli is a daily activity in many molecular biology laboratories. A number of protocols and media recipes have been reported in an effort to make this process more efficient. Here we describe a growth medium that supports much higher E. coli cell densities and, concomitantly, a much higher yield of plasmid than previously reported for small-scale applications. On a unit volume basis, E. coli cultures grown i this medium, termed H15, produce up to 30-fold more recombinant plasmid than in conventional rich media, paralleling the increase in cell density. This phenomenon is independent of E. coli host strain, DNA insert size and plasmid copy number. H15 medium is also very economical; as much as 6 m of plasmid can be harvested per dollar of medium.

Publication Types:
- Technical report

PMID: 9526655 [PubMed - indexed for MEDLINE]

1: Anal Biochem 1989 Feb 1;176(2):344-9

A new method of plasmid DNA preparation by sucrose-mediate detergent lysis from Escherichia coli (gram-negative) and Staphylococcus aureus (gram-positive).

Saha B, Saha D, Niyogi S, Bal M.

Department of Physiology, University College of Science and Technology, India.

A simple and cheap method of plasmid DNA preparation from both gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli) organism is presented here. In this method, in place of the high-priced chemicals lysostaphin and lysozyme which are commonly used for removal c cell-wall during plasmid DNA preparation from gram-positive and gram-negative bacteria, respectively, only sucrose has been used. Firstly, bacteria i treated with Trizma (pH 8.0) containing 100% sucrose (hypertonic solution). Due to this osmotic shock, protoplasm covered by the plasma membrane of bacteria possibly shrinks and becomes detached from the cell-wall. Osmotically sensitive cells thus formed, from gram-positive (S. aureus) and gram-negative (E. coli) bacteria, are finally lysed by the lysis mixture, containing brij 58 and sodium deoxycholate. The lysate is centrifuged at 15,000 rpm for 30 min to pellet the cell debris. The supernatant containing plasmid DNA is treated with either polyethylene glycol or isopropanol. The precipitate which contains plasmid DNA is dissolved in a buffer containing Tris, EDTA, NaCl, and sodium dodecyl sulfate (pH 8.0); thus protein is denatured and removed. Finally, RNA is removed by RNase treatment. The average yield of staphylococcal plasmid DNA as well as plasmid pBR322 from E. coli HB101 in 100% sucrose-treated preparations is greater than that of lysostaphin- and lysozyme-treated preparations. This method is applicable for both large-scale and small-scale preparations. The substrate activity for restriction enzyme, cloning, transforming ability, and electron microscopic profile of the plasmid DNA prepared by this method remains unaltered.

PMID: 2545109 [PubMed - indexed for MEDLINE]

1: Prep Biochem 1985;15(3):121-31

Related Articles, Bool

The enrichment of plasmid DNAs, in bacterial cell lysates, using an alkaline-pH procedure that does not permanently denature them.

Ranhand JM.

The method described permits the enrichment of large (greater than or equal to 54 kb), small (less than or equal to 2.1 kb), or intermediate sizes of plasmi DNAs. It is a modification of the plasmid enrichment method described by Currier and Nester (Anal. Biochem., 76, 431-441, 1976) in that it defers the alkali denaturation step until the pH and temperature can be controlled. This prevents permanent alkali denaturation of some plasmids. In general, total cellular nucleic acids are precipitated with either ethanol or isopropanol after lysates, in 3% w/v NaCl, are extracted with a phenol-chloroform mixture. Th nucleic acids are then treated at an alkaline-pH (12.3-12.4), in a buffer, at 0 degree C, for a minimum time of 15 min. Denatured DNA, in 3% w/v NaCl, is removed with phenol. The RNA- containing, plasmid enriched fraction, is once again precipitated with either ethanol or isopropanol, dried in a vacuum and redissolved. Samples are then digested with various restriction enzymes and/or examined directly on agarose gels after treatment with RNAase A.

PMID: 3932994 [PubMed - indexed for MEDLINE]

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1: Anal Biochem 1996 Oct 15;241(2):186-9

Identification and eradication of a denatured DNA isolated during alkaline lysis-based plasmid purification procedures.

Sayers JR, Evans D, Thomson JB.

University of Sheffield, Section of Molecular Medicine, Krebs Institute, Royal Hallamshire Hospital, United Kingdom. j.r.sayers@sheffield.ac.uk

Many plasmid isolation procedures use strongly alkaline conditions in the initial stages to facilitate lysis of the host bacteria. We demonstrate that such procedures can give rise to a minor but significantly altered form of plasmid. After electrophoresis and uv transillumination of ethidium bromide-stained agarose gels we and others have noticed a faint band migrating near to the major fluorescent product, covalently closed circular plasmid DNA. This fait band is resistant to cleavage by restriction endonucleases which have recognition sites in the parent plasmid. We were able to show that the contaminating band is able to transform competent Escherichia coli cells and that normal double-stranded plasmids were isolated from such transformants. We were able to selectively hydrolyze the contaminating band using T5 exonuclease which is a 5'-nuclease with a single-strand specific endonucleas activity. Plasmid preparations carried out under nonalkaline conditions failed to produce the contaminant band. We suggest methods for purifying plasmid DNA which remove the denatured band and could improve cloning efficiencies where the largest recombinant libraries are required.

PMID: 8921185 [PubMed - indexed for MEDLINE]

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1: Anal Biochem 1986 Feb 1;152(2):215-20

Related Articles, Book

A new rapid procedure for the preparation of plasmid DNA.

Edwardson PA, Atkinson T, Lowe CR, Small DA.

This report describes a simple and efficient procedure for the isolation of plasmid DNA free from chromosomal DNA, cellular RNA, and protein. The technique comprises a modified cleared lysate procedure of D.B. Clewell and D.R. Helinski (1969, Proc. Natl. Acad. Sci. USA, 62, 1159-1166) followed by high-performance liquid chromatography on a Dupont Bioseries GF250 surface stable diol-coated silica gel permeation column (Zorbax) for the final purification of the plasmid DNA. The use of HPLC facilitates rapid and high resolution separations within 3-4 h. Plasmid DNA produced in this manner retains its biological activity and exhibits yields equal to those obtained by the conventional cesium chloride-ethidium bromide density centrifugation method.

PMID: 3516003 [PubMed - indexed for MEDLINE]

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1: J Biochem Biophys Methods 1999 Jul 28;40(1-2):57-64 Related Articles, Book

Contaminant eluted from solid-phase plasmid affinity-purification protocol columns is not found using liquid-phase methods and can be prevented.

Limor R, Gilad S, Kutikof E, Jaffe A, Tendler Y, Gazit V, Stern N, Weisinger G.

Institute of Endocrinology, Tel Aviv Sourasky Medical Center, University of Tel Aviv, Israel.

The preparation of high quality plasmid DNA is a necessary requirement for most molecular biology applications. We compared four different large plasmid preparation protocols, which were based on either a liquid-phase approach (Triton lysis) or purification of alkaline lysis bacterial extracts followed by supercoiled plasmid purification on affinity columns. Two host Escherichia coli strains, JM 109 and INValphaF', were used to grow the test plasmids for comparison of product plasmid DNA produced from the four different plasmid isolation methods. While the DNA grown in E. coli strain JM109, prepared by liquid-phase Triton lysis was appropriately restricted by 12 restriction enzymes, this was not the case for any of the JM109-grown DNA purified by any of the affinity column solid-phase approaches. In contrast to this, when the plasmid DNA was grown in E. coli strain INValphaF', most restriction enzymes cut DNA appropriately, irregardless of the plasmid preparation protocol used. It seems that an impurity commonly eluted with the DNA from all three of the solid-phase DNA columns had an equal effect on the above enzymes using the common host strain JM109, but not strain INValphaF'.

PMID: 10481953 [PubMed - indexed for MEDLINE]

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1: Bioseparation 2000;9(1):1-6

Anion exchange purification of plasmid DNA using expanded bed adsorption.

Ferreira GN, Cabral JM, Prazeres DM.

Centro de Engenharia Biologica e Quimica, Instituto Superior Tecnico, Lisboa, Portugal.

Recent developments in gene therapy with non-viral vectors and DNA vaccination have increased the demand for large amounts of pharmaceutical-grade plasmid DNA. The high viscosity of process streams is of major concern in the purification of plasmids, since it can cause high back pressure in column operations, thus limiting the throughput. In order to avoid these high back pressures, expanded bed anion exchange chromatography was evaluated as an alternative to fixed bed chromatography. A Streamline 25 column filled with 100 ml of Streamline QXL media, was equilibrated with 0.5 M NaCl in TE (10 mM Tris, 1 mM EDTA, pH = 8.0) buffer at an upward flow of 300 cmh-1, E. coli lysates (obtained from up to 3 liters of fermentation broth) were injected in the column. After washing out the unbound material, the media was allowed to sediment and the plasmid was eluted with 1 M NaCl in TE buffer at a downward flow of 120 cmh-1. Purification factors of 36 +/- 1 fold, 26 +/- 0.4 plasmid purity, and close to 100% yields were obtained when less than one settled column volume of plasmid feed was injected. However, both recovery yield and purity abruptly decreased when larger amounts were processed-values of 35 +/- 2 and 5 +/- 0.7 were obtained for the recovery yield and purity, respectively, when 250 n of feedstock were processed. In these cases, gel clogging and expansion collapse were observed. The processing of larger volumes, thus larger plasmi quantities, was only possible by performing an isopropanol precipitation step prior to the chromatographic step. This step led to an enhancement of the purification step.

PMID: 10840595 [PubMed - indexed for MEDLINE]

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1: Anal Biochem 1981 Jul 1;114(2):235-43

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Purification of supercoiled DNA of plasmid col E1 by RPC-5 chromatography.

Best AN, Allison DP, Novelli GD.

PMID: 6272596 [PubMed - indexed for MEDLINE]

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1: Anal Biochem 1983 Dec;135(2):345-8

Related Articles, Book

A simple and rapid procedure for the purification of plasmid DNA using reverse-phase C18 silica beads.

Sparks RB, Elder JH.

A simple and efficient procedure for the rapid isolation of plasmid DNA free of chromosomal DNA and with only minor contamination with RNA is described. The protocol is a modification of the boiling method described by Holmes and Quigley [(1981) Anal. Biochem. 114, 193-197.] and utilizes C18 reverse-phase silica beads for final concentration and purification of plasmid DNA. The entire procedure can be carried out in 1 day and does not require the use of phenol or cesium chloride gradients, which require considerable labor and may sometimes cause nicking and lower recoveries of supercoiled DNA. The plasmid DNA obtained by this method retains biological activity, is supercoiled, and is suitable for restriction and DNA sequence analysis.

PMID: 6318601 [PubMed - indexed for MEDLINE]

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1: J Chromatogr 1982 May 7;240(1):155-63 Related Articles, Bool

Rapid method for purification of plasmid DNA and DNA fragments from DNA linkers using high-performance liquid chromatography on TSK-PW gel.

Himmel ME, Perna PJ, McDonell MW.

High-performance size exclusion chromatography (HPSEC) using TSK-G5000 PW in Tri buffer has been found to be a reliable method for the rapid fractionation of DNA ligation products. Plasmid and fragmented phage DNA were found to elute in less than 2 min with recoveries greater than 98%. Escherichia coli transfection studies, using plasmid DNA that had been subjected to HPSEC column fractionation, showed high transformation efficiencies. MgCl2, a component of the DNA ligation reaction, was found tc produce DNA-column support interactions, which resulted in low DNA recoveries. Such interactions were eliminated by chelation with ethylenediaminetetraacetate prior to chromatography.

PMID: 7047547 [PubMed - indexed for MEDLINE]

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1: Biochem Int 1986 Jun;12(6):889-96

Related Articles, Book

A rapid and inexpensive method for preparing E. coli plasmid-DNA.

Monstein HJ, Geijer T.

A simple, rapid and inexpensive scaled up miniprep procedure for preparing pure E. coli plasmid DNA is described. Bacterial cells were subjected to the boiling procedure and high molecular weight RNA was removed by LiCl-precipitation. Residual RNA and proteins were removed by subsequent treatment with RNase A and proteinase K/SDS respectively, followed by Sephadex G-50 and Sepharose 6B-Cl chromatography. The average yield from a 100 ml over-night bacterial suspension was 100 to 150 micrograms fc pBR-322 DNA, and 250-500 micrograms for SP-6 derived recombinant plasmids. In addition, the described "scaled up" preparation does not require CsCl-ethidium bromide centrifugation; pure plasmid DNA can be prepared within 1 to 2 days.

PMID: 3017351 [PubMed - indexed for MEDLINE]

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1: Gene 1987;54(2-3):255-9

A simple procedure for large-scale purification of plasmid DNA.

Gomez-Marquez J, Freire M, Segade F.

Departamento de Bioquimica y Biologia Molecular, Facultad de Biologia, Universidad de Santiago, Santiago de Compostela, Spain.

We report a simple, rapid and reliable procedure for large-scale purification c plasmid DNA from non-amplified bacterial cultures. It is a modification of the boiling method of Holmes and Quigley [Anal. Biochem. 114 (1981) 193-197] and involves gel-filtration chromatography using Sephacryl S-1000 for final purification of plasmid DNA. This method does not require CsCl gradients and the recovered plasmids are free of RNA and chromosomal DNA, are supercoiled, retain their biological activity, and are suitable for restriction analysis.

PMID: 3308638 [PubMed - indexed for MEDLINE]

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1: Anal Biochem 1989 Mar;177(2):378-82

Purification of plasmid DNA by fast protein liquid chromatography on superose 6 preparative grade.

McClung JK, Gonzales RA.

Samuel Roberts Noble Foundation, Inc., Biomedical Division, Ardmore, Oklahoma 73402.

We were able to reduce both the time and the use of hazardous chemicals associated with the previous plasmid isolation methods of high-pressure liqui chromatography and CsCl gradient centrifugation by employing fast protein liquid chromatography (FPLC). Plasmid was first crudely prepared from bacterial cultures by a standard alkaline lysis method. After an alcohol precipitation, the nucleic acids were divided into two equal portions. One hal was used for a standard purification method employing CsCl centrifugation. The other was dissolved in FPLC buffer, treated with RNase A, and applied t a Superose 6 preparative grade column (HR 10/30). Plasmid eluted off the column within 20 min as a single, highly resolved peak. Plasmid isolated by FPLC had yields, purity, and transformation efficiencies similar to that isolated by CsCl centrifugation.

PMID: 2658679 [PubMed - indexed for MEDLINE]

1: Biotechniques 1991 Jul;11(1):18, 20, 22-4

Purification of plasmid and high molecular mass DNA using PEG-salt two-phase extraction.

Cole KD.

Center for Chemical Technology, National Institutes of Standards and Technology, Boulder, CO 80303.

A method for the rapid preparation of DNA is described. The method utilizes a polymer (polyethylene glycol) and salt solution to form a two-phase system A crude source of DNA is added to a phase-forming mixture, it is mixed and phase separation occurs. Under the appropriate conditions, the nucleic acids remain in the lower (salt-rich) phase, while the proteins, cellular debris and other constituents are in the upper phase (polymer-rich) or are precipitated at the interphase region. Incorporation of protein denaturants (detergents and chaotropes) stop the action of liberated nucleases in the sample. The nucleic acids are obtained in an intact state and in a form suitable for further manipulation, as shown by gel electrophoresis and DNA restriction digestion This method describes the conditions of the two-phase systems that are important for the separation of nucleic acids and proteins. The important phase-forming conditions shown in this paper are pH, polymer molecular weight and concentration, salt type and concentration and the addition of detergents and chaotropic agents. With the use of these extraction conditions proteins can be moved selectively from the lower to the upper phase. The paper describes a method for DNA isolation that is rapid, simple and economical.

PMID: 1954011 [PubMed - indexed for MEDLINE]

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1: Anal Biochem 1992 May 15;203(1):169-72 Related Articles, Bool

Large-scale purification of plasmid DNA by fast protein liquid chromatography using a Hi-Load Q Sepharose column.

Chandra G, Patel P, Kost TA, Gray JG.

Molecular Biology Department, Glaxo Inc. Research Institute, Research Triangle Park, North Carolina 27709.

The large-scale purification of plasmid DNA was achieved using fast protein liquid chromatography on a Hi-Load Q Sepharose column. This method allows for the purification of plasmids starting from crude plasmid DNA, prepared by a simple alkaline lysis procedure, to pure DNA in less than 5 h. I contrast to the previously described plasmid purification methods of CsCl gradient centrifugation or high-pressure liquid chromatography, this method does not require the use of any hazardous or expensive chemicals. More than 100 plasmids varying in size from 3 to 15 kb have been purified using this procedure. A Mono Q Sepharose column was initially used to purify plasmid smaller than 8.0 kb; however, a Hi-Load Q Sepharose column proved more effective with plasmids larger than 8 kb. The loading of plasmids larger than kb on the Mono Q column resulted in a high back pressure and the plasmid DNA could not be eluted from the column. Thus, for routine purification we utilize the Hi-Load Q Sepharose column. Plasmids purified by this method had purity, yield, and transfection efficiency in mammalian cells similar to those of plasmids purified by CsCl density gradient centrifugation.

PMID: 1524212 [PubMed - indexed for MEDLINE]

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